

What is claimed is:

1 1. A carbonyl reductase having the following physicochemical properties:

2 Reactivity

3 It reduces 4-haloacetoacetate ester to produce (S)-4-halo-3 hydroxybutyrate ester
4 using reduced beta-nicotinamide adenine dinucleotide as an electron donor.

5 Substrate specificity

6 It has high reductase activity for 4-chloroacetoacetate ester but does not substantially
7 dehydrogenate any optical isomers of 4-halo-3-hydroxybutyrate ester and

8 shows higher enzymatic activity when used with reduced beta-nicotinamide adenine
9 dinucleotide as an electron donor than reduced beta-nicotinamide adenine dinucleotide
10 phosphate.

1 2. The carbonyl reductase of claim 1, which has additional physicochemical
2 properties below:

3 Optimal pH

4 5.0 to 6.0

5 Substrate specificity

6 It does not substantially dehydrogenate isopropanol and does not reduce acetoacetate.

7 Molecular weight

8 About 32,000 when determined by sodium dodecylsulfate-polyacrylamide gel
9 electrophoresis.

1 3. A substantially pure polypeptide comprising the amino acid sequence represented
2 by SEQ ID NO: 2 and having the enzymatic activity for catalyzing the reduction of 4-
3 haloacetoacetate ester to (S)-4-halo-3-hydroxybutyrate ester using reduced beta-nicotinamide
4 adenine dinucleotide as an electron donor.

1 4. A substantially pure polypeptide comprising the amino acid sequence represented
2 by SEQ ID NO: 2 containing up to 30 conservative amino acid substitutions, and having the
3 following enzymatic activities:

4 reduces 4-haloacetoacetate ester to produce (S)-4-halo-3-hydroxybutyrate ester using
5 reduced beta-nicotinamide adenine dinucleotide as an electron donor;

6 has high reductase activity to 4-chloroacetoacetate ester but does not substantially
7 dehydrogenate any optical isomers of 4-halo-3-hydroxy-butyrate ester; and

8 shows higher enzymatic activity when used with reduced beta-nicotinamide adenine
9 dinucleotide as an electron donor than reduced beta-nicotinamide adenine dinucleotide
10 phosphate.

1 5. A substantially pure polypeptide encoded by a nucleic acid that hybridizes with the
2 nucleic acid consisting the nucleotide sequence represented by SEQ ID NO: 1 under stringent
3 conditions, and having the following enzymatic activities:

4 reduces 4-haloacetoacetate ester to produce (S)-4-halo-3-hydroxybutyrate ester using
5 reduced beta-nicotinamide adenine dinucleotide as an electron donor;

6 has high reductase activity for 4-chloroacetoacetate ester but does not substantially
7 dehydrogenate any optical isomers of 4-halo-3-hydroxybutyrate ester; and

8 shows higher enzymatic activity when used with reduced beta-nicotinamide adenine
9 dinucleotide as an electron donor than reduced beta-nicotinamide adenine dinucleotide
10 phosphate.

1 6. The substantially pure polypeptide of claim 5, comprising an amino acid sequence
2 having at least 70% homology with the amino acid sequence represented by SEQ ID NO: 2.

1 7. An isolated nucleic acid encoding the polypeptide of claim 3.

1 8. An isolated nucleic acid encoding the polypeptide of claim 4.

1 9. An isolated nucleic acid encoding the polypeptide of claim 5.

1 10. An isolated nucleic acid encoding the polypeptide of claim 3 comprising the
2 nucleotide sequence represented by SEQ ID NO: 1.

1 11. An isolated nucleic acid hybridizing with the nucleic acid consisting of the
2 nucleotide sequence represented by SEQ ID NO: 1 under stringent conditions, and encoding a
3 polypeptide having the following enzymatic activities:

4 reduces 4-haloacetoacetate ester to produce (S)-4-halo-3-hydroxybutyrate ester using
5 reduced beta-nicotinamide adenine dinucleotide as an electron donor;

6 has high reductase activity for 4-chloroacetoacetate ester but does not substantially
7 dehydrogenate any optical isomers of 4-halo-3-hydroxybutyrate ester; and

8 shows higher enzymatic activity when used with reduced beta-nicotinamide adenine
9 dinucleotide as an electron donor than reduced beta-nicotinamide adenine dinucleotide
10 phosphate.

1 12. The nucleic acid of claim 11 comprising a nucleotide sequence having at least
2 70% homology with the nucleotide sequence represented by SEQ ID NO: 1.

1 13. A recombinant vector comprising the nucleic acid of claim 7.

1 14. A recombinant vector comprising the nucleic acid of claim 8.

1 15. A recombinant vector comprising the nucleic acid of claim 9.

1 16. A transformant carrying the vector of claim 13.

1 17. A transformant carrying the vector of claim 14.

1 18. A transformant carrying the vector of claim 15.

1 19. The transformant of claim 16, which is a microorganism.

1 20. A method for producing a carbonyl reductase, the method comprising culturing
2 the transformant of claim 16.

1 21. A recombinant vector comprising the nucleic acid of claim 7 and the nucleic acid
2 encoding a glucose dehydrogenase.

1 22. The vector of claim 21, wherein a glucose dehydrogenase is derived from *Bacillus*
2 *subtilis*.

1 23. A transformant carrying the vector of claim 21.

1 24. The transformant of claim 21, which is a microorganism.

1 25. A method for producing an enzyme, the method comprising culturing a
2 microorganism belonging to the genus *Kluyveromyces* and producing the enzyme of claim 1.

1 26. The method of claim 25, wherein the enzyme comprises the amino acid sequence
2 represented by SEQ ID NO: 2.

1 27. The method of claim 25, wherein the microorganism belonging to the genus
2 *Kluyveromyces* is *Kluyveromyces aestuarii*.

1 28. A method for producing a polypeptide, the method comprising culturing the
2 transformant of claim 16.

1 29. The method of claim 28, wherein the transformant is a microorganism.

1 30. A method for producing alcohol, the method comprising reacting ketone with the
2 carbonyl reductase of claim 1, microorganisms producing it, or treated microorganisms.

1 31. The method of claim 30, wherein the carbonyl reductase comprises the amino acid
2 sequence represented by SEQ ID NO: 2.

1 32. A method of producing alcohol comprising reacting ketone with the transformant
2 of claim 16.

1 33. The method for producing alcohol of claim 30, wherein ketone is a derivative of
2 4-haloacetoacetate ester, and alcohol is a derivative of (S)-4-halo-3-hydroxybutyrate ester.

1 34. The method for producing alcohol of claim 33, wherein the derivative of ethyl 4-
2 haloacetoacetate is 4-chloroacetoacetate ester, and alcohol is ethyl (S)-4-chloro-3-
3 hydroxybutyrate.

1 35. The method for producing alcohol of claim 30, the method further comprising
2 converting oxidized beta-nicotinamide adenine dinucleotide to its reduced form.

1 36. The method for producing alcohol of claim 35, wherein oxidized beta-
2 nicotinamide adenine dinucleotide is reduced by a conversion of glucose to delta-
3 gluconolactone by using a glucose dehydrogenase.

1 37. The method for producing alcohol of claim 36, wherein glucose dehydrogenase is
2 expressed by the transformant.